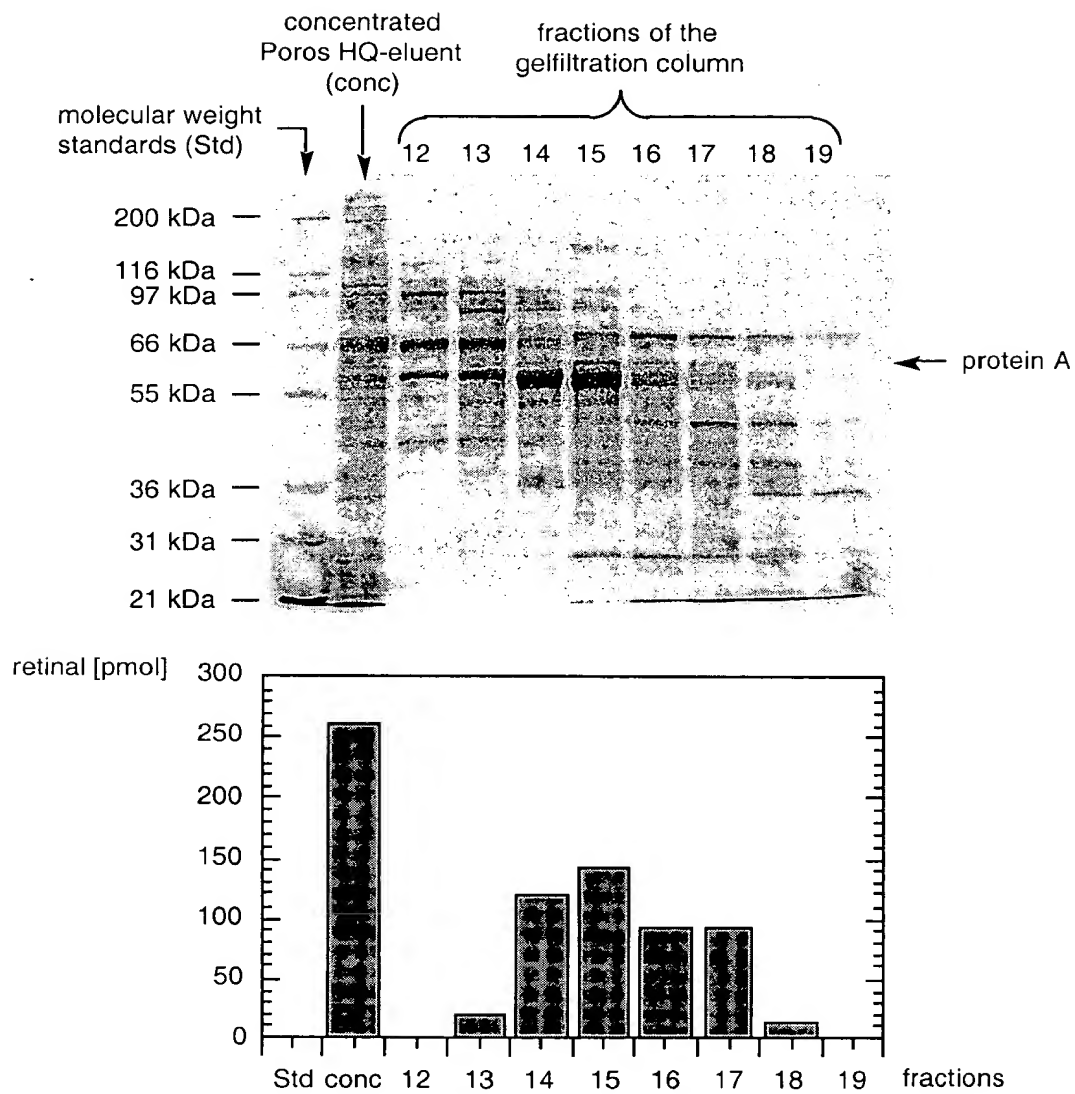


Figure 1



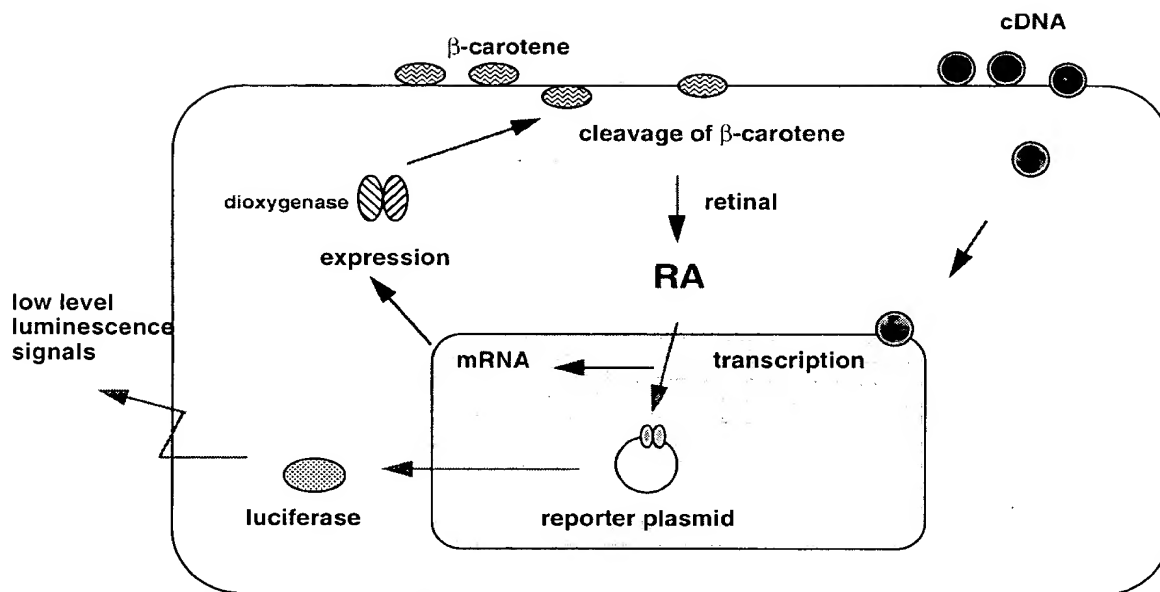


Figure 2

Figure 3
No. 2

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1 CGGATCCACT AGTAACGGCC GCCAGTGTGG TGGAATCCAT
CCTTCTATGT

51 AACAGGAAAG AGCTGTTCTT AGCCCAGAGA GGAGGGCACC
GTACGCCTGC

101 AGGAGCAGCT GGGTAGAGGA CACAGGAGAG CGATGGAGAC
AATATTTAAC

151 AGAAACAAAG AAGAGCATCC AGAGCCCATA AAAGCTGAGG
TGCAAGGTCA

201 GTTGCCCACT TGGTTGCAAG GGGTACTTCT CCGAAATGGC
CCAGGGATGC

251 ACACAATAGG GGACACTAAA TACAACCACT GGTTCGATGG
CTTGGCTCTG

301 CTGCACAGCT TCACGTTTAA AAATGGTGAA GTTTACTACA
GAAGTAAGTA

351 CCTCCGAAGT GACACATACA ACTGCAATAT AGAAGCAAAC
CGAATCGTGG

401 TGTCTGAGTT TGAACCATG GCTTATCCGG ATCCATGCAA
AAACATATTT

451 GCCAAGGCAT TCTCATACTT ATCTCACACC ATTCCTGAGT
TCACGGACAA

501 CTGCCTGATC AACATTATGA AAAGTGGGGA TGATTATTAT
GCTACCAGTG

551 AGACTAACTT CATCAGAAAA ATTGATCCAC AGACTCTGGA
GACACTAGAT

601 AAGGTAGACT ACAGCAAATA TGTAAGCTGTA AACTTGGCAA
CTTCTCACCC

651 ACACTATGAC AGTGCTGGAA ATATTCTCAA CATGGGTACT
TCAATTGTTG

701 ATAAAGGGAG AACAAAATAT GTTCTCTTTA AGATCCCTTC
CTCTGTACCA

751 GAAAAAGAAA AGAAGAAATC TTGTTTTAAA CACCTGGAAG
TAGTATGCTC

801 CATCCCTTCT CGCTCCCTGC TCCAACCAAG CTAATACCAC
AGCTTTGGAA

851 TCACAGAAAA TTATATTGTG TTCATAGAGC AGCCATTTAA
ACTGGATATT

901 GTCAAAGTGG CAACTGCCTA CATCCGAGGT GTGAACTGGG
CTTCCTGCCT

951 TTCCTTTCAT AAGGAGGATA AGACGTGGTT TCACTTTGTA
GACAGAAAGA

1001 CGAAAAAAGA AGTATCCACC AAGTTTACAC CTGATGCTTT
GGTGCTTTAT

1051 CACCACATAA ATGCTTACGA AGAAGATGGC CACGTTGTTT
TTGATATCGT

1101 TGCCTACAGA GACAATAGCT TGTACGATAT GTTTTACTTA
AAAAAACTGG

1151 ACAAAGACTT TGAAGTGAAC AACAAAGCTTA CCTCCATCCC
AACCTGCAAG

1201 CGCTTTGTTG TGCCTCTGCA GTATGACAAG GATGCAGAAG
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1251 TTTAGTCAAA CTTCCAACCTT CCGCAACTGC TGTAAGAGAA
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1301 GCATCTATTG TCAACCTGAA ATATTATGTG AAGGGATAGA
ACTGCCTCGT

1351 GTCAAATATG ACTACAATGG CAAAAAATAC AAGTATGTCT
ATGCAACAGA

1401 AGTCCAGTGG AGCCCAAGTTC CTACAAAGAT TGCAAAACTG
AATGTCCAAA

1451 CAAAGGAAGT ACTGCACTGG GGAGAAGACC ACTGCTGGCC
CTCAGAGCCC

1501 ATCTTTGTTC CCAGCCCCGA TGCAAGAGAA GAGGATGAAG
GTGTTGTTTT

1551 GACCTGTGTT GTGGTGTCTG AGCCAAATAA AGCACCCTTC
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1601 TGGATGCTAA AACATTCAAA GAATTGGGCC GAGCCACAGT
TAACGTAGAA

1651 ATGCATCTGG ACCTGCATGG GATGTTTATA CCACAGAATG
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1701 TGAGACGGAA TAAAACGCTA TTGATCCGAC TACACAACT
GAGACAACCT

1751 TCTACTGAAC ATGAGTTAAT ATCCCTTTTA CCATTCAAGA
ACAACCATAT

1801 AACGACACAA AATGACTATG TATAATCTCT TAAATAATAG
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1851 TTTAAGGCAC AGCGATGAGT TTTACTACAG GTAACGATAT
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1901 CATATAACTA TTCCAAAAGA AGAAGAACGA TCAGTGTTTT
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1951 ATGTTGTACA TAACGGCGGC AGAGGGAACA GGAGAGAAAG
GTAACGGGAA

2001 TATTTAATAG AATATAGATT TCTGAGCAA TGAAGTGCAG
TATTTATGGT

2051 GTGATGCATG GCATGAGTCA CATAGGTCTG CAGCTCATGT
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2101 GATCGTTTCA AGATTGCAGC TTGTGATGCA AGTTTTCTCC
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2151 CCTCATTTTA AACCATCTGC TACTGGTAAT TCATACCAAT
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2201 GGTGCTCGAT TTACACTATA ACCAAAGTTA AGTATTACAT
TCAGGTGCTA

2251 CAACTTTCTA ATTTACAACC GAAACAAACA AGCAAACAGC
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2301 CTAATAACCC CATGGTGTAT TTTTCCTTTT TATGATGACA
AAACCAAGTA

2351 CATATGGTTT TATGTAGCAT TCAATTATAC TTCAGTGCTA
TTCCATCCTA

2401 ATGTTATAAG CAATTTGTAT TTAAATCAGT TTTCCTTGAG
AATATCTGAC

2451 ATAACATTTT GTGTAATGAG ATGACTATGT TGTCTAAAGA
TGAACAGGAA

2501 TGTATCTTTT ATTAGTATTG TTAATTGTGT TACTAATACT
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2551 ATGAGAGCAA TGTATTTCTA GGAGAACTCA GATATACATT
CAACAATTTT

2601 TGTAGGTGAA AATGCATTTA CTGATGAAAG TTGAATCGTT
AATGAGGGAG

2651 AAAACTGGGT ATCCATCCAT CCAACTATGT TAGGTGTTCA
CCTGGTCTGT

2701 ATGTGACACC ACGCTGTTTG GGTATCTCTC ACTTTCACAT
ACCTGTTCTC

2751 ATGGTTTCTG CTACTCACTG TATTTTGCAG GAGAGAAACA
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2801 ACTGTCACCT ACTATCGCCC CATCACATAA GAACAATGGG
GCTTTGGTGA

2851 CTTGTTTCATG ATTACATAAG ATGTTTGCAG CAGAGCAGCA
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2901 CACCATCCAC AGTTCTTGCT TGCTCTGTTA TGACTCCCTT
TGCTGTCTTT

2951 ATGGTTTGCA TGTATGAAGA ATACACTGCC TAATTCTAAT
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3001 CACTGGGGTC AGATCTAGAG CTTAAGTAAG CAGTCTGGGG
TTTTCAAATG

3051 TTTATATGTT CCATAAAATG GAAATAAACA CCTCCATAAT
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Figure 4
No. 1

Seq. ID

1 METIFNRNKE EHPEPIKAEV QGQLPTWLQG VLLRNGPGMH
TIGDTKYNHW

51 FDGLALLHSF TFKNGEVYYR SKYLRSDTYN CNIEANRIVV
SEFGTMAYPD

101 PCKNIFAKAF SYLSHTIPEF TDNCLINIMK TGDDYYATSE
TNFIRKIDPQ

151 TLETLDKVDY SKYVAVNLAT SHPHYDSAGN ILNMGTSIVD
KGRTKYVLFK

201 IPSSVPEKEK KKSCFKHLEV VCSIPSRSLI QPSYYHSFGI
TENYIVFIEQ

251 PFKLDIVKLA TAYIRGVNWA SCLSFHKEDK TWFHFVDRKT
KKEVSTKFYT

301 DALVLYHHIN AYEEDGHVVF DIVAYRDNLS YDMFYLLKLD
KDFEVNNKLT

351 SIPTCKRFVV PLQYDKDAEV GSNLVKLPTS ATAVKEKDGS
IYCQPEILCE

401 GIELPRVNYD YNGKKYKYVY ATEVQWSPVP TKIAKLVNQT
KEVLHWGEDH

451 CWPSEPIFVP SPDAREEDEG VVLTCTVVVSE PNKAPFLIL
DAKTFKELGR

501 ATVNVEMHLD LHGMFIPQND LGAETE

Figure 5

Seq ID No. 4 and Seq ID No. 5

10 EEHPEPIKAEVQGQLPTWLQGVLLR..NGPGMHTIGDTKYNHWF DGLALL
57
20 EELSSPLTAHV TGRIP LWTG SLLRCFTGPG LFEVGSEPFYHLFDGQALL
69
58 HSFTFKNGEVYYRSKYLRSDTYNCNIEANRIVVSEFG..TMAYPD PCKNI
105
70 HKFDFKEGHV TYHRRFIRTDAYVRAMTEKRIVITEFGFTTCAFPDPCKNI
119
106 FAKAFSYLSHTIPEFTDNCLINIMKTGDDYATSETNFIRKIDPQTLETI
155
120 FSRFFSYFRGV..EVT DNALVNVYPVGEDYYACTETNFITKINPETLETI
167
156 ..DKVDYSKYVAVNLATSHPHYDSAGNILNMGTSIVDKGR TKYVLFKIPS
203
168 FTKQVDLCNYVSVNGATAHPHIENDGT VYNIGNCFGKNFSIAYNIVKIPP
217
204 SVPEKEKKKSCFKHLEVVC SIPSRSL LQPSYYHSFGITENYIVFIEQPFK
253
218 LQADKEDPISKFTS.EIVVQFP CSDRFKPSYVHSFGLTPNYIVFVETPVK
266
254 LDIVKLATAY.IRGVNWASCL.SFHKEDK.TWFHFVDRKTKKEVSTK FYT
300
267 INLFKFLSSWSLWGANYMDCFESFTNETMGVWLHIADKKRKKYLNNKYRT
316
301 DALVLYHHINAYEEDGHVVFDIVAYRDN SL...YDMFY LKKLDKDFE...
344
317 SPFNLFHHINTYEDNGFLIVDLCCWKGF EFVYNYFTLYLANLRENWEEVK
366
345 VNNKLTSIPTCKRFV VPLQYDKDAEVGSNLVKLP.TSATAV..KEKDGS I
391
367 KNARKAPQPEVRRYVLPLNIDK.ADTGKNLVTLPNTTATAILCSDEFTTI
415
392 YCQPEILCEG....IELPRVNYD.YNGKKYKYVYATEVQWSPVPTKIAKL
436
416 WLEPEVLFSGPRQAFEPQIN YQKYCGKPYTYAYGLGLNHF.VPDR LCKL
464

4

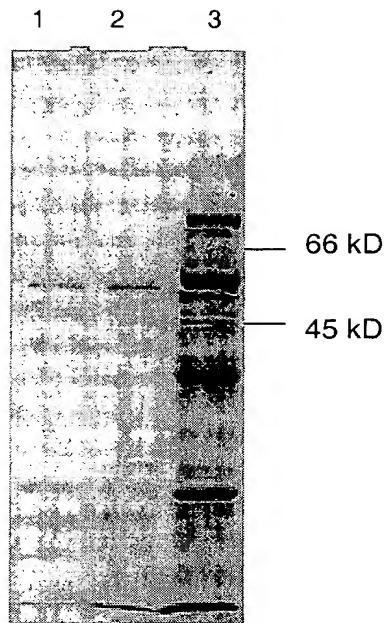


Fig. 6 shows a 10% polyacrylamide gel with E.coli expressed β,β -carotene 15,15'-dioxygenase after affinity tag purification; lane 1 and lane 2: 2 fractions from the Co^{2+} -chelate column showing the main band at 60 kD; lane 3: low range molecular weight marker (Bio Rad).

CHROMATOGRAPH

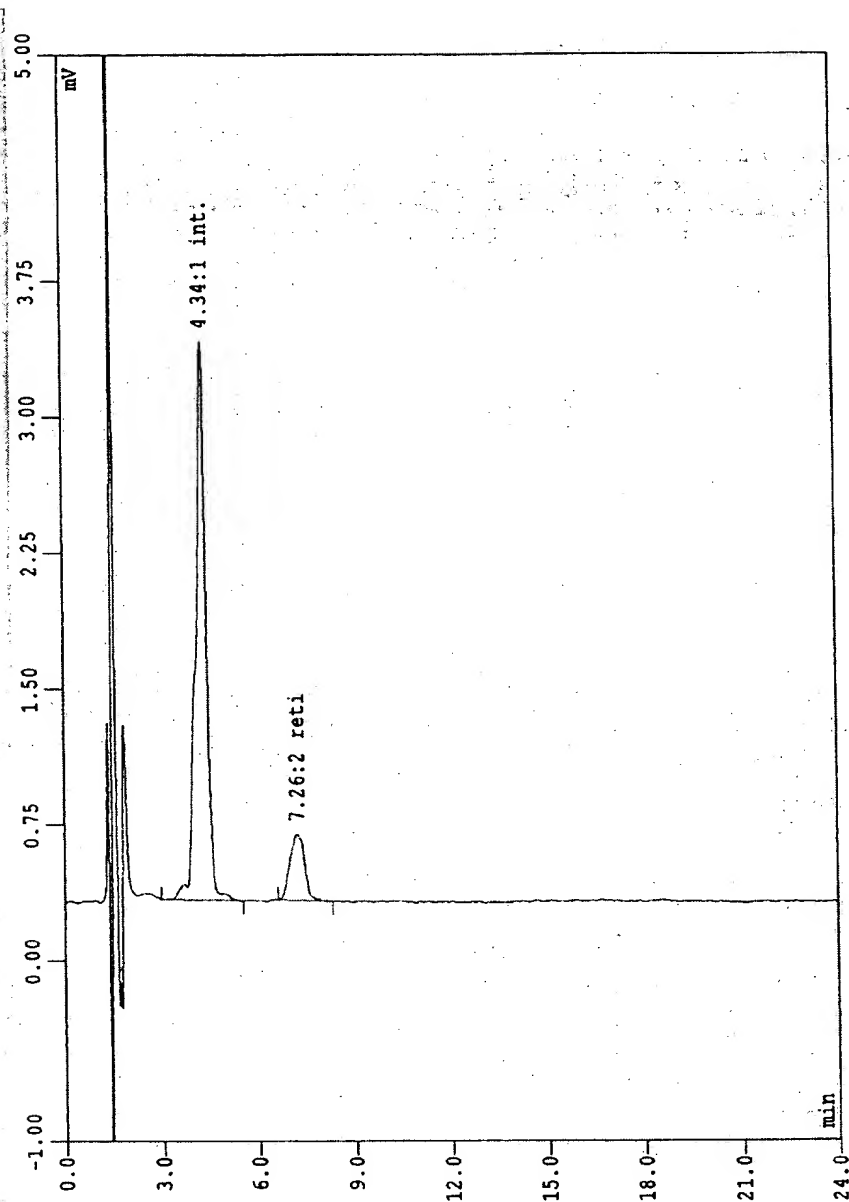


Fig. 7 shows an HPLC profile of the reaction mixture at the end of an activity assay for the β,β -carotene 15,15'-dioxygenase following the procedure in example 1. The first peak in the chromatogram represents the internal standard, while the second peak corresponds to retinal as the only product formed during the central cleavage with β -carotene as substrate.

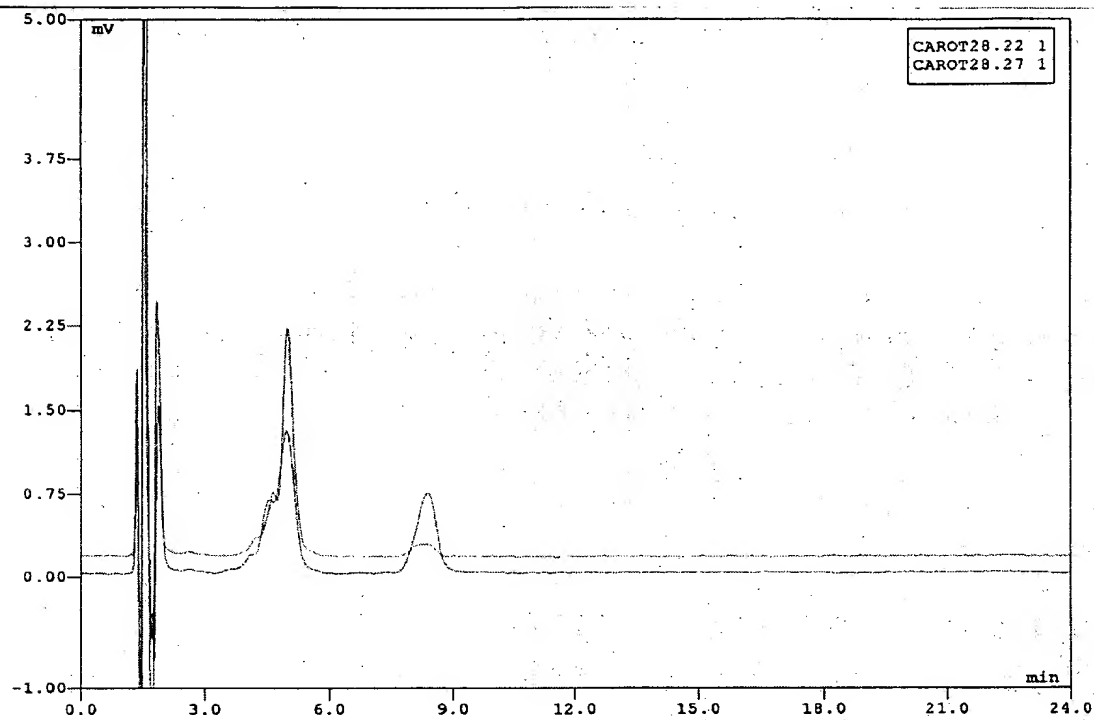


Fig. 8 confirms that the product peak in Fig. 7 is indeed retinal. A sample which was positive in the activity assay (green chromatogram) was spiked with retinal and analysed in second HPLC run (red chromatogram). The chromatograms of the two runs were then overlayed.